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The Commercial Use of Azadirachtin and Its Integration into Viable Pest Control Programmes†

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Abstract: With the continued robust growth of the global biopesticide market, azadirachtin is uniquely positioned to become a key insecticide to expand in this market segment. In the USA the actual or impending cancellation of some organophosphate and carbamate insecticides that have either lost patent protection or are not being re-registered in many markets because of the Food Quality Protection Act of 1996, has opened new opportunities for biopesticides and reduced-risk pesticides in general. The broad-spectrum activity of azadirachtin at low use rates (12·5 to 40 g AI ha^{-1}) coupled with the insect growth regulator activity (in all larval/nymphal instars including the pupal stage) and unique mode of action (ecdysone disruptor), make azadirachtin an ideal candidate for insecticide resistance, integrated pest control and organic pest control programmes.

Azadirachtin has been exempted from residue tolerance requirements by the US Environmental Protection Agency for food crop applications. Azadirachtin exhibits good efficacy against key pests such as whiteflies, leafminers, fungus gnats, thrips, aphids and many leaf-eating caterpillars. Azadirachtin has minimal to no impact on non-target organisms, is compatible with other biological control agents and has a good fit into classical Integrated Pest Management programmes.

The world's largest azadirachtin extraction facility has been fully commissioned in India to process over 10,000 tonnes neem seeds per annum. This will ensure the wide availability of azadirachtin technical grade material in the future.

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1 INTRODUCTION

The Indian neem tree (*Azadirachta indica* A. Juss) has long been recognized as a source of valuable plant allelochemicals with uses including dental (bark extracts), antifungal and antibacterial applications from leaf extracts.[1] However, it is the insecticidal and insect-repellent properties of neem kernel extracts which have attracted worldwide attention.[2] The key active ingredient is azadirachtin, a nortriterpenoid which exhibits classical insect growth regulator (IGR) effects on the immature stages of insects. While the effects of azadirachtin such as antifeedant, reproductive and IGR on many species of insects are well understood, the molecular mechanisms of action are still being elucidated.[3]

The insecticidal properties of neem have been recognized for a long time among the farmers in the Indian sub-continent. Neem leaves were used to control or repel stored-grain pests over centuries. However it has been the growing movement for sustainable agriculture and the need for more biorational products that have stimulated azadirachtin and biopesticides in general, as suitable alternatives to traditional pest control approaches. During the early development years (1970s), the extraction and formulation technology was

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very primitive.[4] Sophisticated extraction processes now exist that can efficiently produce technical grade materials for packaging into various types of stable formulations.

1.1 History

Vikwood Industries was the first company to register a neem formulation in the United States in July 1985. W. R. Grace acquired this technology and marketed 'Margosan O', a 0·3 g AI litre^{-1} azadirachtin EC formulation. Subsequently, a 2·5 g AI litre^{-1} EC was introduced during the early 1990s, followed by a 45 g AI litre^{-1} in 1995 that is currently marketed as 'Neemix' and 'NeemAzad'. The same company also developed neem oil 'Triact' for the control of insects as well as various pathogenic fungi. In 1996, W. R. Grace sold the biopesticide unit to ThermoTrilogy (a subsidiary of ThermoEcotek Inc.). In 1992, another neem manufacturer, AgriDyne Technologies, received registration to market a 30 g AI litre^{-1} azadirachtin formulation ('Azatin', 'Turplex' and 'Align') and a 45 g AI kg^{-1} dihydroazadirachtin (a hydrogenated azadirachtin derivative) formulation, in 1996. With the merger of AgriDyne Technologies with Biosys during October 1995 and the subsequent purchase of Biosys by ThermoTrilogy, both azadirachtin registrations in the US currently belong to ThermoTrilogy. As the largest basic producer of azadirachtin, Fortune Biotech Limited is the most recent player to enter the global market. Fortune Biotech entered into an exclusive arrangement with American Vanguard (AmVac) in November 1996 to develop and distribute Fortune's azadirachtin products into the US markets.

2 KEY STEPS IN THE COMMERCIALIZATION PROCESS

2.1 Raw material supply

Perhaps one of the most significant hurdles that had to be cleared was the availability of quality raw material for azadirachtin extraction. Although native to the Indian subcontinent, neem trees are widely spread through the tropics. The drought-hardy nature of the neem tree has made it an ideal candidate for reforesting arid areas in many tropical countries. However, despite the tree's widespread occurrence, India is perhaps the only country with the infrastructure already in place for procuring large quantities of raw material (seed). During the late 1970s, mainly due to acute shortage of edible oils, the Government of India provided many incentives to the solvent-extraction oil industry to utilize non-edible oilseeds and make such oils available to the soap and shampoo manufacturing industries, thereby lessening their dependence on edible oils. Neem oil was the oilseed of choice, not only because of widespread availability at the village level, but also because of the high oil content (>40%) in the kernel. Neem seeds (or fruit) are hand-picked from the ground, depulped and sun-dried in the villages and consolidated into tonne-lot quantities before being shipped to the nearest auction centre. Seeds arrive at the market between July and October each year. Oil-mill operators process the seeds through mechanical expellers and/or solvent extractors to produce the neem oil. Currently, there are 141 seed collection and 70 oil production centres in the country.[6] It is estimated that over 125 000 tonnes of neem seed are collected and sold in these markets annually which accounts for only around 15–20% of the total neem seed potential in India alone.

Azadirachtin content in the kernel peaks at fruit maturity, and rapid fermentation of the pulp upon fruit drop reduces the azadirachtin content. By encouraging seed collectors at the village level to depulp freshly dropped fruit at various locations, Fortune Biotech Limited has ensured that at least one-third to one-half of its raw material requirement will come directly from primary sources, bypassing the auction houses. Additional depulping units are expected to be established in the future so that the entire manufacturing is based on directly supplied raw material that is processed with minimal azadirachtin loss.

2.2 Manufacturing

On arrival to the factory, the seeds are cleaned and mechanically dried down to 10–12% moisture level before storing in ventilated silos. When stored properly, there is minimal decomposition of azadirachtin. If seeds are improperly dried, mould development is encouraged and fungi such as *Aspergillus flavus* contaminate the seed lots with aflatoxins.[7] Therefore, proper handling and storage of the raw materials is critical in the manufacturing process. The neem seeds are fed from the silos into a decortication unit (separation of the hulls and kernels) and the kernels proceed into a mechanical expeller to provide a 'soft press'. This step results in the production of some oil and an oil-rich cake. By using proprietary solvent extraction technology, the azadirachtin is now extracted from the cake as a yellowish-brown powder containing 12–26% azadirachtin. This is the technical grade material that is used to make a wide range of formulations. As part of the quality control process, steps are taken to ensure that the aflatoxin content does not exceed 15 parts per billion in the formulation grade technical product.

Fortune Biotech's goal is to manufacture around 10 tonnes azadirachtin from 10 000 tonnes neem seeds in a dedicated, state-of-the-art facility in Hyderabad, India. This output is expected to be sufficient to treat 0·5 to 0·75 million ha. The factory has been fully commis-

sioned and began commercial production in August 1997.

2.3 Formulation and storage stability

The technical powder containing 12–26% azadirachtin (higher concentrates can be manufactured but at disproportionately higher cost) is versatile for making a wide range of formulations. These technical powders themselves have acceptable storage stability, losing only 3–5% of the azadirachtin content over 1 year when stored at room temperature and low humidity. This facilitates shipping and handling. The creation of storage-stable formulations, particularly ECs, has, however, presented a challenge. In fact, the lack of shelf life among the early azadirachtin formulations was one of the main reasons for the erratic efficacy results. However, it was only during the last decade that serious efforts have been made to develop stable EC formulations. At present, there are several patents with procedures and compositions that claim to confer unique stability to azadirachtin in ECs.[8] In general, the higher the azadirachtin concentration in the final formulation, the greater the instability. All else being equal, formulations having less than 10 g $litre^{-1}$ have flatter degradation curves than formulations containing 20 g AI $litre^{-1}$ or more. Manufacture of azadirachtin formulations with good stability and dispersion characteristics is essential for grower acceptance and usage, especially in high-value markets. Formulations with poor stability profiles generally provide unpredictable and ineffective insect control.

2.4 Value of by-products

In the overall manufacturing process, all the by-products obtained have valuable commercial uses in India. The hulls are used as a soil amendment and are often incorporated back into the de-oiled (solvent-extracted) neem cake. The neem cake is sought after by tea and coffee estate managers, where it is used extensively (and often exclusively) as a fertilizer and a bio-nematicide. Neem oil is in demand by soap and shampoo manufacturers as well as for insecticidal and medicinal uses. The export of neem oil from India has grown dramatically over the past few years. It should be noted that the cost of seed in the auction houses has gone up substantially, from US$ 115 per tonne three to four years ago to around US$ 200 per tonne today. One tonne of neem seed yields approximately 0·75–1·5 kg AI azadirachtin, 220 kg neem oil, 380 kg neem cake and approximately 400 kg hulls. It is also important to note that, given the high cost of seeds and extraction for azadirachtin, establishing markets for the by-products is essential to offset the manufacturing costs. In India, neem oil retails for around US$ 675 per tonne, and neem cake fetches around US$ 95 per tonne. However, it is the production cost that ultimately determines the type of market in which azadirachtin can compete with other biological and synthetic insecticides.

3 AZADIRACHTIN MARKETS

There are over 200 pest species belonging to the major insect orders of Diptera, Hymenoptera, Coleoptera, Lepidoptera, Orthoptera, Homoptera and Hemiptera that are susceptible to the effects of azadirachtin.[1] However, only a few of these pests can be considered commercial targets because the relatively higher cost of production (compared with synthetic insecticides) restricts azadirachtin to high-value markets or to areas where growers are willing to pay a premium for pest control.

3.1 Growth of biopesticides

Lisanky and Coombs[9] estimated that the annual growth rate of biopesticides was 10%. Growth has occurred mainly in high-value niche markets. The global biopesticide insecticide market is around US$ 90 million today with *Bacillus thuringiensis* (Bt) products accounting for almost 80% of the market share. Azadirachtin and other biopesticides are gaining ground mainly for the following reasons:

(1) The cancellation of older registrations and the lack of support for re-registration due to loss of patent protection;
(2) Companies opting not to register newer products into smaller niche markets where the risk of failure and product liability is also generally higher;
(3) Insect resistance and cross-resistance to current compounds;
(4) Government mandates to cut synthetic insecticide use rates. For example, the US Congress passed a law (the Food Quality Protection Act; FQPA) in August 1996 which will, for example, combine exposures from all organophosphate insecticides into the same 'risk cup' when validating the food tolerance of any given organophosphate insecticide. This could potentially lead to numerous withdrawals of existing insecticides, particularly from smaller niche markets;
(5) Elimination of biological control agents, such as parasites and predators, by synthetic insecticides, leading to secondary pest outbreaks.
(6) Lower cost and faster approval of biopesticide registrations coupled with tighter and increased regulations for the newer synthetic insecticides;

(7) Improved and consistent product performance from biopesticides as technologies advance;
(8) Consolidation of agrochemical companies leading to a cutback on synthetic chemical R&D, resulting in fewer new products in the pipeline;
(9) Continued consumer awareness on pesticide safety issues and shifting trends towards use of biorationals.

Azadirachtin, which has been classified as a biorational insecticide by the US-EPA, is exempt from residue tolerance requirements on food crops as long as the dosage does not exceed 50 g AI ha^{-1} per application (49 CFR, 180.1119). Azadirachtin is already registered in the US and has been in the market for over eight years. Recently Spain and Sweden have approved azadirachtin for sale based on a similar biopesticide classification as in the US. Many Latin American countries, Nicaragua, Costa Rica, Dominican Republic, Colombia, Guatemala among others, have approved azadirachtin for both food and non-food uses. Fortune Biotech's azadirachtin is currently being evaluated by both EPA and California-EPA and registrations are expected in July 1998.

Many biopesticides such as Bt, entomopathogenic nematodes (*Steinernema* spp), live biocontrol agents, such as insect parasitoids, *Beauvaria* spp, etc. or even semiochemicals such as pheromones are characterized by having a narrow spectrum of control, thereby restricting their applications to a specific pest and related species. While this specificity is desirable from an insect pest management (IPM) perspective, it presents a marketing challenge because it often requires tailoring the product (different strain, type etc.) for specific markets, resulting in multiple products – each with specific applicability. Therefore, the major advantage of azadirachtin is that the same product can be applied as part of an IPM program against different pest spectra in different niche markets with similar good results. The suggested application rates for azadirachtin generally range from 12·5 g to 40 g AI ha^{-1} and it can therefore be considered as one of the most active insecticidal molecules.

Some of the markets in which azadirachtin has a strong position are outlined in the following sections.

3.1.1 *Greenhouse, ornamentals and interiorscape*

Azadirachtin has a unique fit in this market segment. Target pests include: whitefly (*Bemisia argentifolii* Bellows & Perring), *Trialeurodes vaporariorum* (Westwood), leafminer (*Liriomyza trifolii* (Burgess)), *Fenusa pusilla* (Lepeletier), thrips (*Frankliniella occidentalis* (Pergande)), certain species of aphid such as *Myzus persicae* (Sulzer) and *Aphis gosypii* Glover, fungus gnat (*Bradysia spp*), mealybug (*Planococcus citri* (Risso)), armyworm (*Spodoptera exigua* (Hubner)), *Agrotis spp* and black vine weevil (*Otiorhynchus ovatus* (L.)). Application rates vary from 20 to 40 g AI ha^{-1}.

3.1.2 *Turf and outdoor ornamentals*

For golf courses and ornamental tree pest control the target pests include lepidopteran larvae such as gypsy moth (*Lymantria dispar* (L.)), spruce budworm (*Choristoneura fumiferana* (Clemens)), cankerworm (*Alsophila pometaria* (Harris)), tent caterpillar (*Malacosoma americanum* (F.)), webworm (*Herpetogramma licarsisalis* (Walker)), leafminer (*Phyllocnistis meliacella* Becker), cutworm (*Agrotis spp*), armyworm (*Spodoptera spp*) sawfly (*Neodiprion sertifer* (Geoffroy)), leafhopper (*Erythroneura elegantula* Osborn), bark beetle (*Dendroctonus spp*), Japanese beetle (*Popillia japonica* Newman), flea beetle (*Epitrix spp*), psylla (*Cacopsylla pyricola* Foerster), and certain scales (e.g. *Saissetia oleae* (Bern.)). Application rates range from 15 to 37·5 g AI ha^{-1}.

3.1.3 *Mushrooms*

This speciality crop market has a unique fit for azadirachtin for fly control, such as mushroom (sciarid) fly (*Lycoriella mali*) and phorid fly (*Megaselia halterata* Wood) on all speciality and *Agaricus* mushroom varieties. Applications are recommended at the rate of 50 g AI ha^{-1} (minimum four drench applications made 7 to 10 days apart).

3.1.4 *Vegetables, herbs, fruit and nut crops*

The key target pests include diamondback moth (*Plutella xylostella* (L.)), cabbage looper (*Trichoplusia ni* (Hubner)), imported cabbageworm (*Pieris rapae* (L.)), leafminers (*Liromyza spp*), Colorado potato beetle (*Leptinotarsa decemlineata* (Say)), bean beetle (*Cerotoma trifurcata* (Forster)), pepper weevil (*Anthonomus eugenii* Cano), peachtree borer (*Synanthedon exitosa* (Say)), pinworm (*Keiferia lycopersicella* (Walsingham)), tobacco budworm (*Helicoverpa virescens* (F.)), tobacco hornworm (*Manduca sexta* (L.)), tomato fruitworm (*Helicoverpa zea* (Boddie)), grapeleaf skeletonizer (*Harrisina brillians* Barnes & McDunnough), cherry maggot (*Rhagoletis cingulata* (Loew)), silverleaf whitefly (*Bemisia argentifolii* Bellows & Perring). Application rates range from 12·5 to 25 g AI ha^{-1}. Addition of a non-phytotoxic crop oil (0·5 to 1% v/v) prolongs residual activity and yields more consistent results (Fortune Biotech, unpublished data).

3.1.5 *Home and garden*

The pest list is a combination of the greenhouse, interioscape, turf and ornamentals and the high-value food crops listed above. A more dilute, ready-to-use product is desirable.

3.1.6 *Organic markets*

The organic market (apples and other fruit, leafy vegetables, grapes and herbs) in the US is growing at an

annual rate of 12 to 15%, following large acreages being certified as organic. Recently the US Department of Agriculture announced a proposed set of federal standards for organic foods developed by the National Organic Standards Board. Current azadirachtin formulations have been approved for use on organic produce. At present, only azadirachtin affords good control of leafminers among the various insecticides available. The target pest complex is similar to the vegetable, herbs, fruit and nut crops.

3.2 Product positioning

Neem extracts affect insects in many ways, demonstrating effects as a repellent, an antifeedant, oviposition inhibitor and sterilant. For the purposes of commercialization it is only the insect growth regulator (IGR) effect that can be exploited because the other effects are either of short duration, affect specific insects only under certain conditions, or are unpredictable. The IGR effect exhibits a good dose-response and is a reliable predictor of efficacy. With the emergence of many synthetic IGRs (such as the benzoylphenylureas, trianines, and, recently, the aryl hydrazines) they have become accepted into mainstream pest control regimens, and the key disadvantage of the slow onset of activity (often taking four to eight days) is now more acceptable to growers. The main point to emphasize with azadirachtin (as with other IGRs) is the fact that there is no adulticidal activity and it can take up to 10 days to observe mortality. Death of insects is typically observed between molts, in the pupal stage or at adult eclosion. It is recommended that at least two or three applications are made seven to 10 days apart to effectively shut down the insect life cycle. However, insects stop feeding long before they die.[10]

3.3 Integration of azadirachtin into IPM and resistance management programs

When working with natural enemies such as parasites and predators either under natural or introduced conditions, azadirachtin is an ideal complementary insecticide in IPM systems because it effectively kills the phytophagous insects while having minimal impact on beneficials.[11] Azadirachtin has little or no activity against mites or adult beneficials, therefore a program combining predatory mites (such as *Ambyleseius* spp) or predators such as ladybird beetles (*Coccinella* spp) along with azadirachtin will be effective. A typical scenario for control of pests such as leafminers (*Liriomyza* spp) in greenhouses would include application of adulticides (such as a pyrethroid) for reducing the size of the initial populations and their progeny, followed by a sequence of two or three applications of azadirachtin (parasitoids such as *Diglyphus* spp may also be incorporated, if needed). This approach minimizes resistance development by using insecticides with different modes of action and possibly different mechanisms of resistance. This strategy also ensures attack on all stages of the insect, widening the window of control. Azadirachtin has little or no impact on pollinators such a honey bees, bumble bees, and other non-target organisms (Fortune Biotech, unpublished data).

4 CONCLUSION

The production of azadirachtin is a labour-intensive and expensive process and, for the foreseeable future, neem seed will remain the only viable source of azadirachtin because of the difficulties in the synthetic approach.[12] However, because of pest control conditions existing in the world today, it is expected that this remarkable and unique biorational insecticide will become more extensively used in many markets worldwide.

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